NEURAL RESPONSES TO INJURY: PREVENTION, PROTECTION, AND REPAIR
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Neuropharmacology of Delta Receptor Agonists and Antagonists

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The experimental animals used during this period for the project, Neural Responses to Injury: Prevention, Protection and Repair, **Subproject: Neuropharmacology of Delta Receptor Agonists and Antagonists**, are as follows:

Species	Number Allowed	Number Used	LSU IACUC #
rhesus monkey	6	6	1062

Investigator Signature

J. M. Moerschbaecher, Ph.D.

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ABSTRACT

Studies in the Division of Neuropharmacology are investigating the role of endogenous opioid systems in learning and memory, ventilatory function and antinociception. The goal of these studies is: to identify and characterize candidate ligands that might be useful in studies on *delta* opioid mechanisms; and to use these compounds to systematically investigate the role of *delta* systems in complex behavioral processes, in respiration and in the perception of noxious stimuli. The first candidate compound was BW373U86, which is a highly-selective agonist for the *delta* opioid receptor. BW373U86 has effects that are unlike effects obtained with prototypic *mu* or *kappa* opioid agonists. This year, the candidate compound which was focused upon was OHM3507. This compound differs from morphine in terms of its effects on the immune system. It was hypothesized that this difference might be due to activity at the delta opioid receptor. The pharmacologic and behavioral effects of the fentanyl derivative OHM3507 were assessed to determine if this compound had increased antinociceptive effects and a reduced number of undesirable effects (e.g., respiratory depression) as compared to the prototypic opioids (e.g., fentanyl, morphine). The current studies were undertaken to determine the opioid receptor selectivity of OHM3507 using behavioral assays in rhesus monkey. At a dose of 0.32 mg/kg, OHM3507 produced 100% antinociception (20s latency) in monkeys and reduced minute volume respiration to <60% in both air and 5% CO_2 in O_2. In subjects treated daily with morphine (3.2 mg/kg) discriminating between saline and 0.01 mg/kg naltrexone, OHM3507 attenuated responding on the naltrexone-associated lever in a dose-dependent manner. OHM3507 decreased the rate of responding at doses greater than 0.1 mg/kg, but did not disrupt learning or performance of a complex behavioral task in monkeys responding under a schedule of food presentation. The administration of naltrexone dose-dependently shifted the

OHM3507 dose effect curve to the right with pA_2s of 7.8 and 8.2 for antinociception and discrimination, respectively. OHM3507 produced 100% antinociception at 3.2 mg/kg. These data suggest that: 1) OHM3507 produces fentanyl-like effects at the μ opioid receptor in rhesus monkeys, in contrast to its effects in rodents; 2) the reported lack of NK-cell activity after OHM3507 administration suggests a reduced risk of immunosuppression produced by this chemical class and 3) there is a necessity to study this class of compounds under a variety of experimental conditions in different species in order to better characterize the properties of these compounds and their potential clinical values, however, our studies indicate that they are devoid of *delta* opioid receptor activity.

INTRODUCTION

The 4-heteroanilido-piperidine class is a class of compounds which seems to be characterized by compounds having novel combinations of opioid and non-opioid receptor mediated effects. One of these derivatives, mirfentanil, has been studied extensively and has low efficacy at μ opioid receptors compared to fentanyl and morphine in rodents. Mirfentanil produces antinociceptive effects and a limited amount of respiratory depressive effects, but reverses morphine-induced antinociception and respiratory depression (Wynn et al, 1994). In contrast, mirfentanil, in rhesus monkeys, produces some antinociceptive effects that do not appear to be mediated by μ, κ, or δ opioid receptors (France, et al., 1991), since its antinociceptive effects not antagonized by naltrexone, and produces little to no respiratory depressant effects (France et al., 1991) suggesting a possible non-opioid mechanisms for this compound.

Another member of this class, OHM3507, is a low efficacy opioid agonist in rodents and rabbits; OHM3507 produces modest antinociceptive effects in the rabbit tooth pulp assay and reverses morphine-induced respiratory depression and antinociception in rabbits (Wynn *et al.*, 1991). This profile of effects is similar to mirfentanil and suggests that OHM3507 may be a novel compound of the same type as mirfentanil, producing antinociception with a decreased amount of respiratory depressant effects, which would increase the therapeutic potential of the compound. However, one characteristic becoming apparent in this class of compounds is the differentiation of effects seen with these compounds in different species. Compound 28 (OHM3568), for example, has low efficacy in producing antinociception in rodents, and has a pharmacological profile nearly identical to fentanyl in non-human primates (France *et al.*, 1992), while OHM3295 is a low efficacy

opioid agonist in non-human primates and in rodents (France *et al.*, 1995). Because of the varying responses obtained among different species with this family of compounds, systemic comparisons among a variety of species appears warranted for these fentanyl derivatives.

In the current studies, the antinociceptive effects of OHM3507 as well is its respiratory depressant effects and amnesic/dissociative effects were determined in rhesus monkeys. The pharmacologic effects of OHM3507 were characterized using a warm-water tail withdrawal procedure to measure antinociception, pressure changes in a head plethysmograph to measure respiration, and the learning and performance of a discrimination under a food schedule to study complex behavior.

MATERIALS AND METHODS

Subjects.

Adult rhesus monkeys (*Macaca mulatta*) were housed individually and given free access to water. The subjects were fed Purina Monkey Chow and received fresh fruit twice weekly. The subjects that were used to measure complex behavior were maintained at 85% of their free-feeding weights by banana-flavored pellets received during experimental sessions and supplemental feeding in the home cage; all other subjects were maintained at their free-feeding weights. In addition, the subjects involved in drug discrimination studies received 3.2 mg/kg/d (s.c.) of morphine. All subjects, except for two in the antinociception study, had been involved previously in opioid studies.

Apparatus. *Antinociception Studies.* Primate restraining chairs made of Plexiglass and aluminum piping were used to loosely restrain the subjects at the neck and waist to allow free access to their tails, which hung unimpeded from the bottom of the seat. Thermos bottles were filled with water of different temperatures (40, 50, 55°C) heated by a hot-water bath. Temperatures were determined to within one degree of the desired temperature using a mercury thermometer. Latency was measured manually by the investigator and recorded using a push-button switch connected to a computer (IBM PCjr).

Respiration Studies. Subjects were seated in primate restraining chairs made of Lexan which were located within a sound-attenuating chamber. Alternating layers of Lexan plates (2) and latex collars (2) as well as a foam cushion formed the base of the plethysmograph to form a seal to minimize gas leakage from the plethysmograph. Air or 5% CO_2 in O_2 was pumped into the plethysmograph at a rate of 10 L/min and removed with a vacuum pump. Changes in air

pressure were measured using a pressure transducer and recorded by a microprocessor (Dell Opiplex 433/L). These values were used to calculate f (frequency in resp/min), V_T (tidal volume in L/resp) and V_e (min volume, L/min).

Drug Discrimination. Subjects were seated in Lexan primate restraining chairs located in sound-attenuating operant chambers that were equipped with two or three response levers and accompanying red stimulus lights located above each lever. Each subject was fitted with a pair of shoes containing brass electrodes, through which brief electric shocks (250 msec) could be administered from an A.C. generator located outside the chamber. Experimental sessions were controlled and data were recorded by microprocessors.

Performance and Acquisition Studies. A removable response panel (69 cm X 22 cm X 47 cm; BRS/LVE, Laurel, MD; model TIP-002) was attached to the side of the home cage (76 cm X 71 cm X 97 cm; Research Equipment Co., Inc., Byran, TX; model LC-1004) during experimental sessions. Three translucent response keys (BRS/LVE, press plate model PPC-012) were located on the response panel 50 cm from the cage floor and 11.5 cm apart. Reinforcers were delivered into an aperture (5.5 cm in diameter) located to the right of the rightmost key. Responses were recorded through a microprocessor (Tandy; Fort Worth, TX) and recording equipment located in an adjacent room.

Procedures.

Antinociception Studies. A warm-water tail withdrawal procedure was used to measure antinociceptive effects (Dykstra and Woods, 1986). The latency for the subject to remove the tail from the warm water (40, 50, or 55°C) was used as a measure of antinociceptive effect. In order to measure latency, monkeys were placed in chairs and the bottom 10-12 cm of

the shaved tail was placed in the water until the subject removed the tail, or until 20s had passed, whichever occurred first.

Control (pre-drug) measurements were taken after the animals had been seated in the chairs for at least 10 min. Drug was administered s.c. on either side of the back or upper arm, alternating sides with each injection. Effective dose ranges and duration of drug effects were determined for morphine and for OHM3507 using single dose studies with 15 min testing intervals (10 min pretreatment, 5 min period for assessing tail withdrawal latencies) for a total session time that did not exceed 90 min. Drugs were administered no more than twice weekly, with an intervening period of at least 48 h between tests.

Utilizing the dosing information determined with single doses, cumulative dosing studies with 30-min interinjection intervals were used in subsequent experiments. When a maximum effect (20s latency) was obtained in all subjects at 50°C, the session was terminated, except during the determination of the time course of OHM3507 when studies were carried out to 100% maximum possible effect in 55°C water. Control dose effect curves for OHM3507 were conducted using both sterile H_2O and a propylene glycol vehicle. In antagonism studies, a single dose of antagonist (naltrindole or naltrexone) was administered s.c. 10-15 min prior to the initial injection of agonist. Since the duration of the measurable effects of naltrexone (0.01 mg/kg) declines after approximately 2.5 h, sessions with antagonists were limited to 90 min, or a maximum of 5 doses of agonist. Control latencies were determined immediately before the administration of antagonist, and again immediately prior to the first agonist injection. For comparison, cumulative dose-effect curves also were determined for morphine, fentnyl, and mirfentayl.

Respiration studies. These studies are a modification of those previously described by Howell *et al.*, 1988 and France *et al.*, 1990. After placing the head plethysmograph on the Lexan/latex base (described above in **Apparatus**), the primate chair was placed within a sound-attenuating chamber. Experimental sessions consisted of a series of successive,discrete 30-min cycles, beginning with a saline cycle (as control) followed by 2-6 cycles of either drug or saline. Each cycle was divided into a 23- min exposure to air, followed by a 7-min exposure to 5% CO_2 in O_2. Injections of saline or drug were given s.c. in the back during the first minute of each cycle. Data were recorded for each minute throughout the cycle, but reported as a mean of the last three minutes of exposure to air and 5% CO_2 in O_2. Drugs were administered no more than twice weekly and separated by an intervening period of at least 48 h.

Cumulative dose- response curves for OHM3507 were generated (using dosing schedules from antinociception studies), by increasing the amount of drug injected by either half- or quarter-log units in successive cycles. Tests were concluded when subjects attained 50% of control respirations per min (rpm), or for 8 cycles, whichever occurred first. During antagonism studies, a single injection of 0.01 mg/kg of naltrexone was administered one cycle prior to the first dose of agonist.

Drug Discrimination. The procedure for studying drug discrimination in morphine-treated subjects under a fixed-ratio schedule of shock termination has been described elsewhere (France and Gerak, 1994). Briefly, subjects received daily s.c. injections of 3.2 mg/kg/day of morphine 3 hr prior to sessions and discriminated between injections of saline and 0.1 mg/kg of naltrexone. Training sessions consisted of multiple 15-min cycles consisting of a 10 min timeout, during which the chamber was dark and responses had no programmed consequence,

followed by a 5-min response period, during which stimulus lights were illuminated and five responses on the appropriate lever (saline- or naltrexone-associated) resulted in the termination of the shock-associated stimulus and postponement of the shock for 30s. Injections (s.c.) were administered during the first minute of the time-out period. Stimuli were terminated after 5 min or 4 shocks, whichever occurred first. Responses on the incorrect lever reset the response requirement on the correct lever. During saline training sessions, saline injections were administered at the beginning of each cycle; during drug training days, 1-5 saline or sham cycles preceded the administration of naltrexone.

Testing conditions were identical to the training sessions, except that 1) saline was substituted for the daily morphine dose 3 h prior to session, 2) five consecutive responses on either lever resulted in the postponement of shock (no lever was designated as correct) and 3) doses of drug were administered under a cumulative dosing schedule with doses increasing by 1/4 log units. For antagonism studies, naltrexone was administered on the first cycle.

Learning and Performance Studies. A multiple schedule of repeated acquisition and performance of conditional discriminations has been described previously (Moerschbaecher and Thompson, 1983) and served as the base-line procedure. In this procedure the effects of a drug can be evaluated on both the acquisition and performance of a discrimination with a single subject within a single experimental session. In each component of the multiple schedule, the task was to respond on either the right or left key depending upon the stimulus displayed on the center key. Correct responses resulted in progression to the next component of the chain in which a different stimulus was displayed on the center key; incorrect responses resulted in a 5-sec timeout during which reponding had no programmed consequences. Completion of a two-

member chain of these discriminations resulted in the delivery of a 500-mg food pellet. During each session a different chain of condtional discriminations was required during one component of a multiple schedule (acquisition/learning component), whereas in the other component the chain of conditional discriminations was the same each session (performance component). The components alternated after 20 food presentations or 15 min whichever occurred first. A 5-sec timeout, during which all stimuli were off and responses had no programmed consequence, separated consecutive components. Sessions terminated after 200 food presentations or 2 hr, whichever occurred first. Sessions were conducted 5 days per week and always began in the acquisition component. Drug sessions were generally conducted on Tuesdays and Fridays (no more than twice per week), and control (saline) injections on Thursdays. Drugs were administered s.c. in the back 10-15 min prior to the session.

Data Analyses.

Percent of maximum antinociception (%MPE) was calculated in the following manner: % MPE = [(experimental latency - baseline latency) ÷ (20 - baseline latency)]. These values were calculated individually for each subject then averaged among subjects. These mean values (± 1 SEM) were plotted as a function of dose or time. Potency comparisons among drugs were estimated by examining differences in ED_{50}s determined by linear regression (three or more points) or interpolation (2 points). Apparent antagonist affinities (pA_2 and pK_B) were estimated using the methods of Arunlakshana and Schild (1959) as well as the Schild analysis plot with slope constrained to -1 (Tallarida *et al.*, 1979). Physiologic changes in the subjects, e.g. flushing, pupillary dilation, decreased activity, were also noted during antinociception studies, and recorded 5 min prior to each testing interval (in 15 min interval studies) or every 15 min in 30

min interval studies.

Respiratory depression was observed using a comparison of known respiratory indices, f, V_T, and V_e. V_e (minute volume) was calculated by multiplying V_T and f. V_e was plotted as a function of dose of drug for individual subjects in both air and 5% CO_2 in O_2.

Drug discrimination data are presented as the percentage of responding on the drug-associated lever (%NTX) and calculated as: [(number of responses on the naltrexone-associated lever) ÷ (total number of responses)] x 100. These data were plotted (± 1 SEM) as a function of dose. Drugs are considered to substitute for the training drug (naltrexone) when they produce >90% responding on the drug-associated lever.

The effects of drugs on acquisition and performance were determined by calculating the overall response rate (in responses/min), and the percentage of errors ([incorrect/total number of responses] x 100%) for the individual componentsof the multiple schedule. The percentage of errors was calculated for each successive block of 10 food presentations for both saline and drug sessions to measure the within-session learning of the task. Comparisons between drug sessions and the control range of variability in saline sessions, were carried out for each subject. A drug dose was considered to have an effect to the extent that the data fell outside of the control range.

Drugs. The drugs used in these studies were morphine sulfate, fentanyl, naltrexone hydrochloride, and naltrindole hydrochloride (National Institute on Drug Abuse, Rockville, MD), and mirfentanyl and OHM3507 hydrochloride (synthesized by L. L. Brockunier according to Bagley *et al.*, 1989; OHMEDA Inc, Murray Hill, NJ). Drugs were dissolved in sterile 0.9% saline or H_2O (OHM3507). For the antagonism studies with concentrations of drug greater than 1 mg/ml, OHM3507 was dissolved in a vehicle solution of 40% propylene glycol, 50%

physiological saline, and 10% ethanol. OHM3507 was made fresh daily as needed.

RESULTS

Antinociception. Monkeys never removed their tails from 40°C water; control tail-withdrawal latencies for 50 and 50°C ranged from 0.38-0.50s. In time course studies, OHM3507 produced both time- and dose-related increases in antinociception **(FIG 1)**. The onset of action was 15 min, and reached a maximum effect between 30 and 75 min. Full (100%) antinociception occurred in all subjects at a dose of 0.32 mg/kg at 50°C and lasted throughout the entire 90-min testing session; 24 h post-injection, values had returned to baseline measurements (data not included), while maximum antinociception occurred 30 min post-injection in 55°C and declined steadily thereafter. Based upon ED_{50}s obtained from the cumulative dose effect curves, the relative potencies were determined to be: fentanyl (0.12 mg/kg) ≥ OHM3507 (0.14 mg/kg) > morphine (1.77 mg/kg) > mirfentanil (7.44 mg/kg).

Naltrexone produced dose-dependent rightward shifts in the OHM3507 dose-effect curve, and significant increases in the OHM3507 ED_{50}s over a dose range of 0.01 to 0.1 mg/kg of naltrexone **(FIG 2-top)**. Schild analysis of naltrexone antagonism yielded a slope of -1.25 and produced a pA_2 of 7.8 **(FIG 2-bottom)**. The pA_2 value with the slope constrained to -1 was 7.81 ± 0.3. Naltrindole (at a dose shown to antagonize agonists acting at the μ receptor [Negus, et al., 1994]), also produced a dose-dependent and rightward parallel shift in the OHM3507 dose effect curve **(FIG 3)**. Calculation of pK_B yielded an affinity estimate of 6.5 for naltrindole.

The propylene glycol vehicle had no effect and redetermination of the OHM3507 dose-effect curve at the end of these studies was not different from the initial OHM3507 curve (data not shown).

Respiration. Morphine produced dose-dependent decreases in VE both air and in 5%

CO_2 in O_2. Effective dose ranges for OHM3507 differed widely; however, a decrease was observed in all subjects at doses ranging from 0.1 to 1.0 mg/kg (**FIG 4**). The respiratory depressant effects of OHM 3507 were antagonized by doses of 0.01 mg/kg naltrexone (data not shown).

Drug Discrimination. In morphine-treated monkeys, both the administration of naltrexone in the presence of morphine and the acute deprivation of morphine (27 hr abstinence) produced 100% responding on the drug (naltrexone)-associated lever (data not shown). OHM3507 dose-dependently reversed naltrexone-associated lever responding, with 0.32 mg/kg completely attenuating responding on the naltrexone-associated lever. This effect was dose-dependently antagonized by the administration of naltrexone, and produced a pA_2 of 8.6 ± 0.2 (**FIG 5**).

Learning and performance. In each of the subjects tested OHM3507 produced rate-decreasing effects (**FIG 6, top panels**) in both the learning and performance components at doses greater than 0.1 mg/kg.. No consistent error-increasing effects in either learning or performance were obtained over a dose range of 0.032- 0.1 mg/kg (FIG 6, Bottom panels). Pretreatment with 0.032 mg/kg of naltrexone, 40 min prior to the session, produced an antagonism of the rate-decreasing effects of OHM3507. A greater than 10-fold shift to the right in the OHM3507 dose response curve was obtained with this dose of naltrexone.

Other Effects of OHM3507. Several behavioral effects, e.g. mydriasis, sedation, flushing of the face, salivation, were observed in most subjects at approximately one-tenth of the doses (0.32 mg/kg) necessary to produce 100% antinociception. There was some indication in the

acquisition and performance studies that OHM 3507 continues to exert some rate-decreasing effects 24 hr or more post-injection. These effects seemed to diminish in intensity with continued exposure to the drug in the antinociception and respiration studies, but not in the study of complex behavioral processes.

CONCLUSIONS

The behavioral and pharmacologic profile of OHM3507 in these studies demonstrates that this compound has both high selectivity and efficacy at the μ opioid receptor in rhesus monkeys, producing effects similar to fentanyl. OHM3507 was evaluated previously in rats, rabbits and mice (Bagley et al., 1989, Wynn et al., 1991) However, the effects of OHM3507 in rodents are not consistent with those seen in these studies in rhesus monkeys.

OHM3507 has a high degree of efficacy at the μ-opioid receptor, producing antinociceptive effects as well as decreasing respiration in both air and 5% CO_2 in O_2. These effects were antagonized by the administration of the opioid antagonist naltrexone, producing rightward shifts in the agonist dose-response curves.

Another measure of the strong μ opioid actions of OHM3507 is its ability to reverse withdrawal in morphine-dependent subjects. Withdrawal can reliably be precipitated in chronically-treated subjects by either the administration of naltrexone, an opioid antagonist, or the deprivation of the daily dose of morphine (France and Woods, 1989; France and Gerak, 1994). In morphine-treated subjects discriminating between injections of saline and naltrexone and acutely deprived of morphine, responding on the naltrexone-associated lever was fully attenuated by OHM3507. This attenuation is similar to that seen with morphine, and other μ-selective opioid agonists.

In addition to some of the observable signs associated with opioid agonist administration (i.e. mydriasis, sedation), OHM3507 also affected complex behavior in a manner similar to morphine (Moerschbaecher and Thompson, 1983). In both learning and performance, the percent of errors produced did not change substantially with the administration of OHM3507

until high doses , where rates of responding decreased greatly. Thus, OHM3507 does not produce selective effects on learning and memory doses lower than those that affect the mechanical performance of these tasks.

Opioid antagonists have been used extensively to differentiate receptor mechanisms through their varying degrees of selectivity and affinity. Naltrexone dose-dependently shifted the OHM3507 dose-effect curve to the right and the pA2s and pK_Bs determined for OHM3507 lie within the dose range established for naltrexone in combination with other μ-selective opioid agonists (France and Gerak, 1994). Additionally, antagonism with naltrindole, at doses that would affect both μ and κ receptors produced a rightward shift in the dose-effect curve; the affinity estimate falls within the range estimates for μ-receptor mediation (Negus et al., 1993).

The purpose of this study was to explore the pharmacological profile of OHM3507, and its potential use as a compound with abilities to produce antinociception, but with reduced adverse effects, based on its profile in other species. However, one characteristic that seems to be emerging about this class of like compounds is the substantial differences seen in physiological and behavioral effects produced with these compounds among species (France et al, 1991; France et al., 1994, France et al, in press). It may be necessary, by using well-established procedures and a variety of species to produce a more accurate pharmacological profile of these compounds in order to better and more accurately predict the possible effects of these drugs in humans, as well as explore the differences that species play in producing the pharmacological effects.

Last year we reported on our studies of BW373U86 using these same methods. While

this compound clearly had greater δ-receptor activity than OHM3507 it was disappointing in terms of its overall pharmacological profile. It exhibited relatively poor systemic activity and significant behavioral toxicity including convulsions and barrel rolling (Comer et al., 1993; Dykstra et al., 1993; Pakarinen et al., 1993). Despite these adverse effects BW373U86 has proven to be an effective lead compound for the development of nonpeptidic δ-opioid agonists. Recently, Bilsky et al. (1995) have reported on the pharmacology of modified enantiomer of BW373U86, SNC 80. They profiled the pharmacology of this compound both in vitro and in vivo. Their data indicated was systemically active and highly selective for the δ-opioid receptor. We hope to focus on this compound in our next series of studies during year 03.

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FIGURE LEGENDS

Figure 1. Time course studies for the antinociceptive effects of OHM3507 using 50°C (upper panel) and 55°C (lower panel) water. N = 4 for all studies, except that of 0.01 mg/kg OHM3507 where N = 3. Ordinates: percent of maximum possible effect (%MPE) ± 1SEM; abscissae: dose in mg/kg body weight.

Figure 2. Antagonism of OHM3507 by naltrexone using a cumulative dosing procedure. Subjects received an injection of naltrexone 10 min before sessions. N = 4 for all groups. See Figure 1 for other details. Lower panel : Schild plot of same antinociception data presented in the upper panel. Ordinate: log(dose ratio - 1); abscissa: -log(dose of naltrexone in moles/kg).

Figure 3. Antagonism of the antinociception effects of OHM3507 by naltrindole. Subjects received 3.2 mg/kg of naltrindole (open squares) prior to cumulative doses of OHM3507. See Figures 1 and 2 for other details.

Figure 4. Dose effect curves of morphine, and OHM3507 on respiration in air and 5% CO_2 using a cumulative-dosing schedule. Values (in air) were taken after injection with saline one session prior to initial injection with drug, and used as baseline. Morphine and OHM3507 results are expressed in terms of V_E (% control) for both air and 5% CO_2 in O_2. Abscissae: dose in mg/kg of body weight; C = control (no drug).

Figure 5. Discriminative stimulus effects of OHM3507 in subjects treated with 3.2 mg/kg/day of morphine and discriminating between injections of saline and naltrexone. For these studies, monkeys received saline, rather than morphine, 3 hrs prior to session. OHM3507 was studied alone (closed circles) as well as in combination with several doses of naltrexone. Ordinate: percent responding on drug-associated lever (%DR); abscissa: dose in mg/kg of body weight.

Figure 6. The effects of OHM3507 on acquisition and performance. Subjects were treated with a dose of drug or vehicle 10 min prior to experimental session. The results of each individual subject are presented (left-Co, middle-B, right-P). Drug data in both the performance (filled circles) and acquisition (open circles) components represent the mean and range of at least two determinations in each subject. The combined effects of OHM3507 and 0.032 mg/kg naltrexone are indicated by triangles (open = acquisition component; closed = performance component). Ordinates: rate in responses/min (upper panels) and percent errors (lower panels); abscissae: dose in mg/kg of body weight.

PUBLICATIONS

Abstracts

Ahn, S.C. Brockunier, L.L., Bagley, J.R., Carr, D.J., Moerschbaecher, J.M. and France, C.P. Comparison of behavioral and immunologic effects of novel fentanyl derivatives. Presented at the Satellite Conference on Aids & Drugs Abuse, College on Problems of Drug Dependence, Scottsdale.

Moerschbaecher, J.M. and Pakarinen, E.D. Effects of Convulsant and Anticonvulsant Agents on Memory in Squirrel Monkeys. Soc. Neuroscience Abstracts 1994, 20: 1021.

Publications

Gerak, L.R., Butelman, E.R., Woods, J.H. and France, C.P. (1994) Antinociceptive and respiratory effects of nalbuphine in rhesus monkeys. Journal of Pharmacology and Experimental Therapeutics, 271, 993-999.

France, C.P., Gerak, L.R., Winger, G.D., Medzihradsky, F., Bagley, J.R., Brokunier, L.L., and Woods, J.H. (1995) Behavioral effects and receptor binding affinities of fentanyl derivatives in rhesus monkeys. Journal of Pharmacology and Experimental Therapeutics, 274, 17-28.

Pakarinen, E.D., Woods, J.H. and Moerschbaecher, J.M. Repeated acquisition of behavioral chains in squirrel monkeys: Comparisons of a Mu, Kappa and delta Opioid Agonist. Journal of

Pharmacology and Experimental Therpeutics, 1995, 272, 552-559.

Pakarinen, E.D., Faust, W.B. & Moerschbaecher, J.M. Effects of convulsant and anticonvulsant agents on memory in squirrel monkeys. Progress in Neuro-Psychopharmacology & Biological Psychiary, 1995 (submitted).

APPENDICES

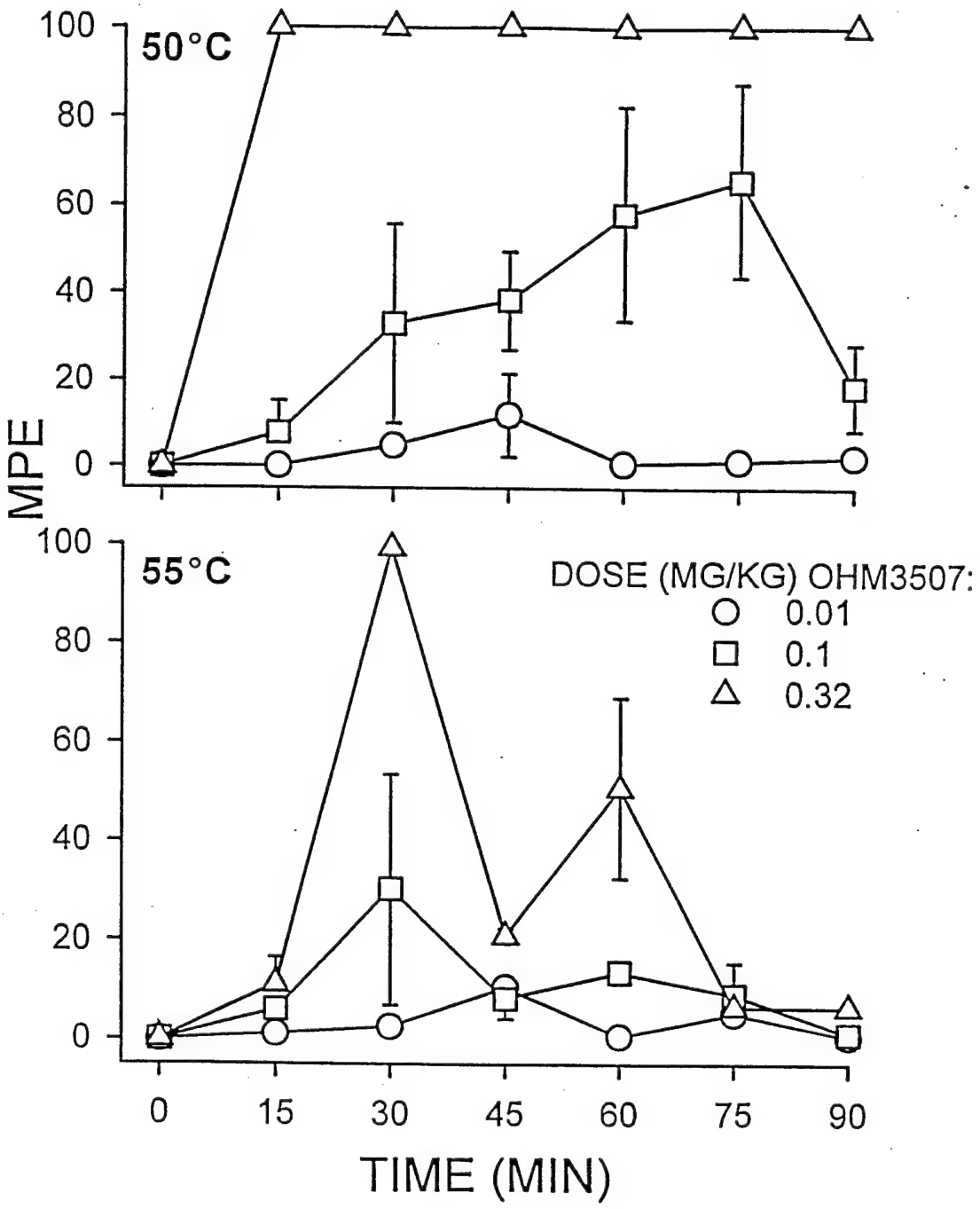
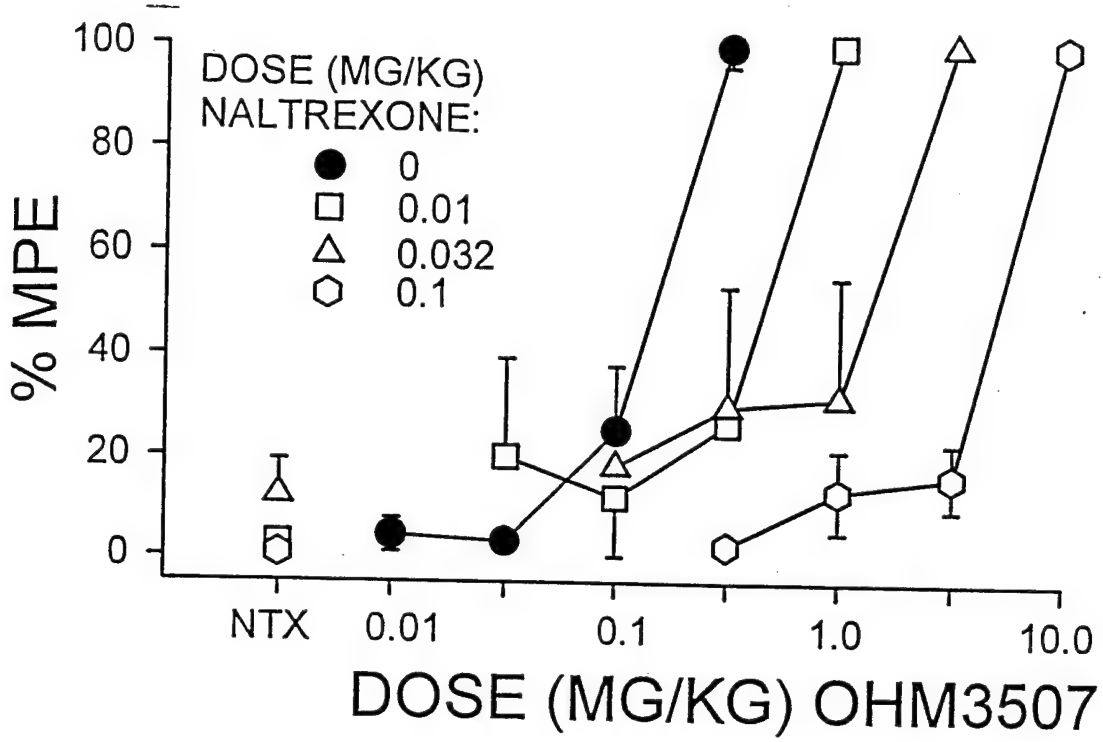

50°C
55°C
MPE
100
80
60
40
20
0
0
15
30
45
60
75
90
TIME (MIN)
DOSE (MG/KG) OHM3507:
0.01
0.1
0.32

Figure 1


100
80
60
40
20
0
% MPE
DOSE (MG/KG)
NALTREXONE:
0
0.01
0.032
0.1
NTX
0.01
0.1
1.0
10.0
DOSE (MG/KG) OHM3507

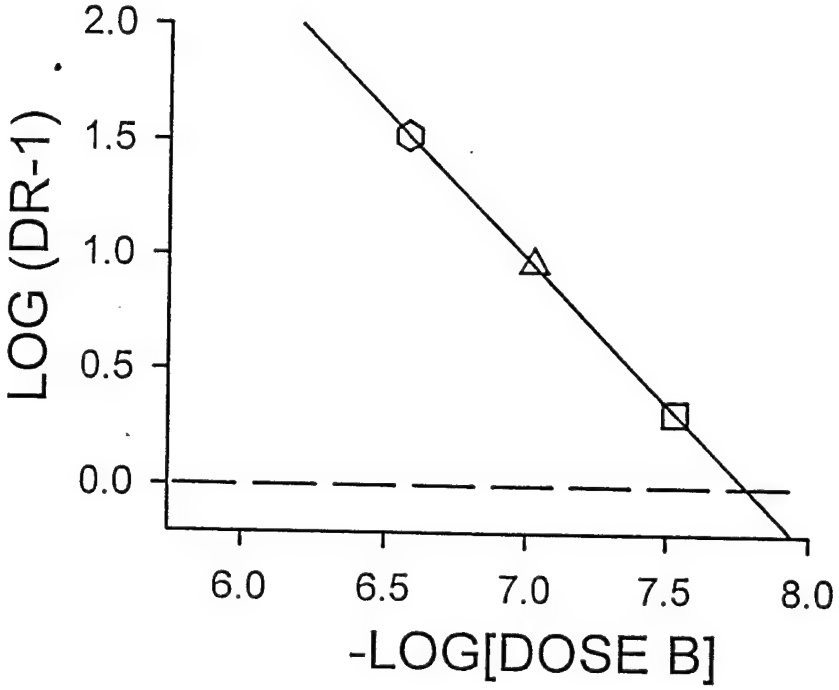

2.0
1.5
1.0
0.5
0.0
LOG (DR-1)
6.0
6.5
7.0
7.5
8.0
-LOG[DOSE B]

Figure 2

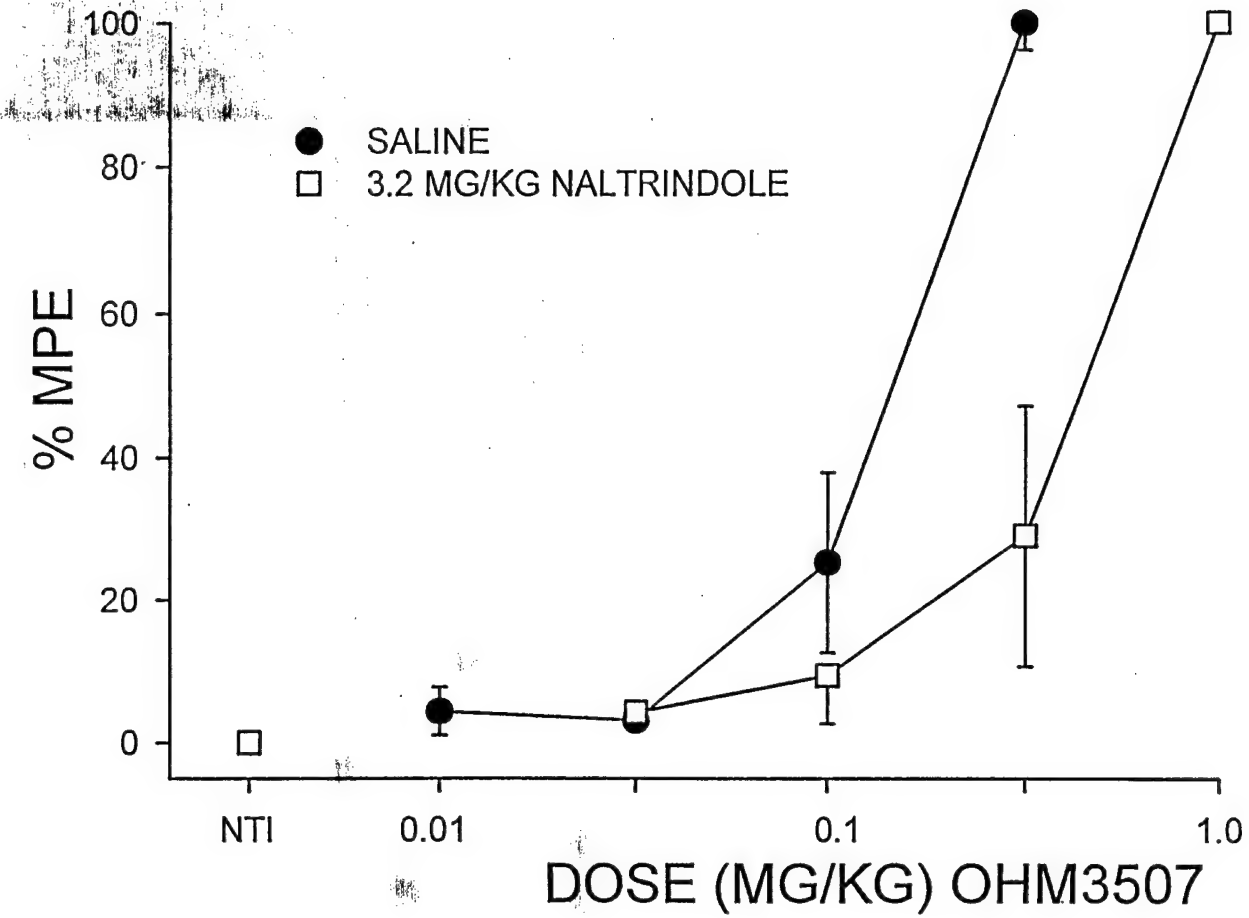

100
80
60
40
20
0
% MPE
SALINE
3.2 MG/KG NALTRINDOLE
NTI
0.01
0.1
1.0
DOSE (MG/KG) OHM3507

Figure 3

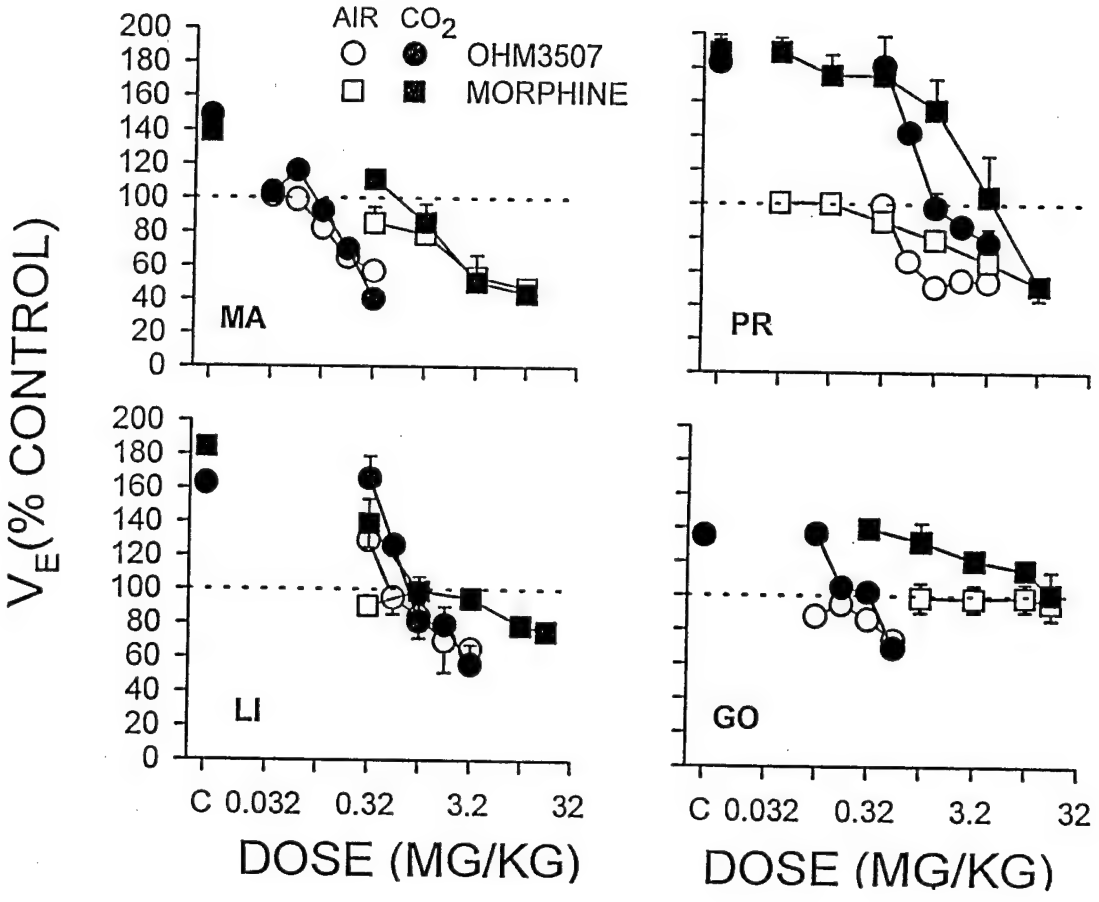

AIR CO2
OHM3507
MORPHINE
MA
PR
LI
GO
VE(% CONTROL)
200
180
160
140
120
100
80
60
40
20
0
C 0.032 0.32 3.2 32
DOSE (MG/KG)

Figure 4

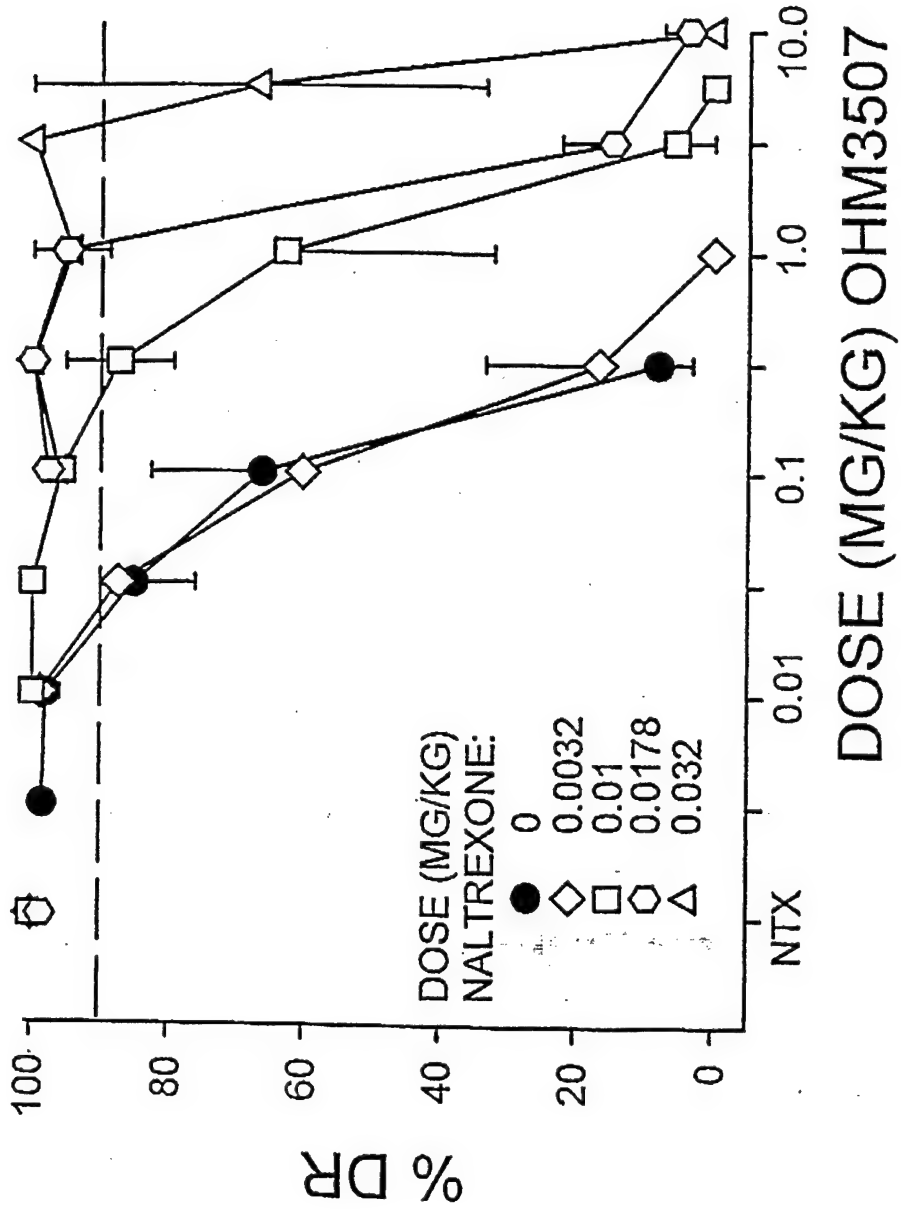

% DR
100
80
60
40
20
0
DOSE (MG/KG)
NALTREXONE:
0
0.0032
0.01
0.0178
0.032
NTX
0.01
0.1
1.0
10.0
DOSE (MG/KG) OHM3507

Figure 5

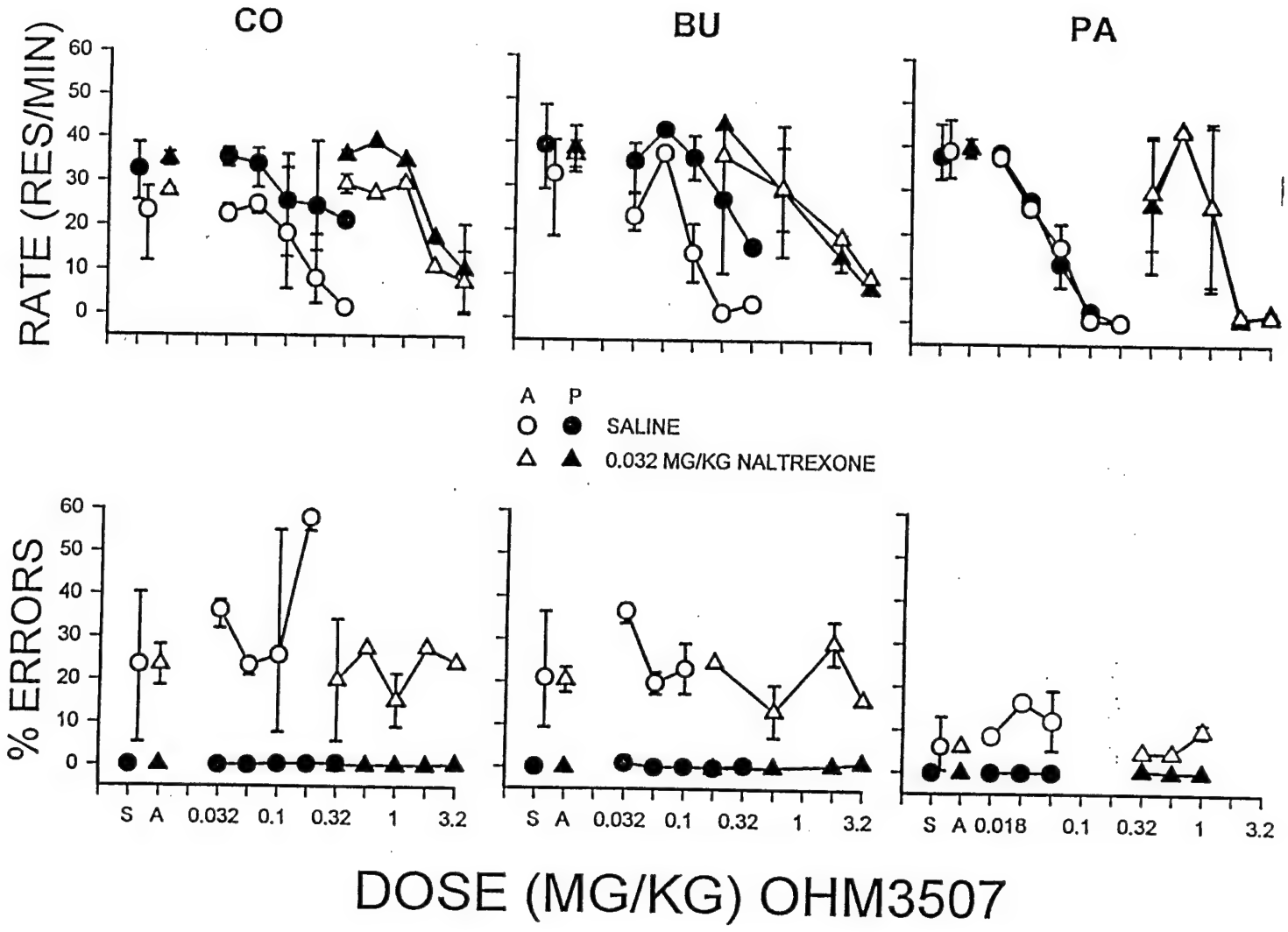

CO
BU
PA
RATE (RES/MIN)
60
50
40
30
20
10
0
A P
SALINE
0.032 MG/KG NALTREXONE
% ERRORS
S A 0.032 0.1 0.32 1 3.2
S A 0.032 0.1 0.32 1 3.2
S A 0.018 0.1 0.32 1 3.2
DOSE (MG/KG) OHM3507

Figure 6

Submitted: European Behavioural Pharmacology Society
Cagliari, Italy; May 17-21, 1996.

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EFFECTS OF NEGATIVE ALLOSTERIC MODULATORS OF $GABA_A$ RECEPTORS ON COMPLEX BEHAVIORAL PROCESSES IN MONKEYS

The effects of positive allosteric modulators of $GABA_A$ receptors on learning and memory have been well studied in recent years, whereas those of negative allosteric modulators or inverse agonists have not received the same attention. For this reason, a multiple schedule of repeated acquisition and performance of conditional discriminations was used to characterize the effects of two inverse agonists (ß-CCE and FG7142), a hallucinogenic ß-carboline derivative (harmine), a benzodiazepine receptor antagonist (flumazenil) and a positive allosteric modulator (alprazolam). In the acquisition component, subjects acquired a different discrimination each session by responding on either the left key or the right key depending upon the stimuli (different colors and geometric forms) presented on the center key. Acquisition of a discrimination was defined by a decrease in errors as the session progressed. In the performance component, the discriminative stimuli for left- or right-key responses were the same each session. Responding in both components of the multiple schedule was maintained by food presentation under a variable-ratio schedule, and each session began with the learning component and then alternated with the performance component. Incorrect responses in both components produced a 5-sec timeout. Alprazolam (0.1-18 mg/kg), ß-CCE (0.01-0.32 mg/kg), FG-7142 (0.1-18 mg/kg) and harmine (0.1-1.8 mg/kg) all dose-dependently decreased response rate in both components. On accuracy of responding, however, the effects depended on the drug administered. Alprazolam selectively and dose-dependently increased percent errors only in acquisition. ß-CCE increased percent errors in acquisition only at the highest dose tested. In contrast, FG 7142 and harmine had no effects on percent errors at doses that virtually eliminated responding. When flumazenil (0.1, 0.32 and 1 mg/kg) was administered alone, no effects were observed on response rate or percent errors in either component, but in combination it dose-dependently attenuated the rate-decreasing effects of ß-CCE, FG7142 and alprazolam, and the error-increasing effects of ß-CCE and alprazolam. In contrast to these effects, flumazenil failed to antagonize the effects of harmine. Thus, the negative allosteric modulators only moderately disrupt acquisition in comparison to the positive allosteric modulators, but the effects of both types of modulator can be antagonized by the $GABA_A$ receptor antagonist flumazenil.

This work was sponsored in part by the Department of the Army, Cooperative Agreement DAMD 17-93-V-3013. This does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred.

1995 Conference on

NIDA
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AIDS and Drug Abuse

PROGRAM AND ABSTRACTS

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roblems of Drug Dependence

P-29
COMPARISON OF BEHAVIORAL AND IMMUNOLOGIC EFFECTS OF NOVEL FENTANYL DERIVATIVES

S.C. Ahn, L.L. Brockunier*, J.R. Bagley*, D.J. Carr**; J.M. Moerschbaecher, and C.P. France
Departments of Pharmacology, and Microbiology and Immunology**, LSUMC, New Orleans, LA and Ohmeda Inc.*, Murray Hill, NJ

These experiments compared the fentanyl derivatives OHM3507 and mirfentanil under several *in vitro* and *in vivo* conditions. In rhesus monkeys, OHM3507: 1) produces a full antinociceptive effect in a warm-water tail withdrawal procedure; 2) markedly decreased respiration in air and in 5% CO_2 in air; 3) does not affect the acquisition or the performance of a complex operant task. Moreover, naltrexone dose-dependently antagonizes the antinociceptive effects of OHM3507, yielding a pA_2 of 7.8. Conversely, mirfentanil does not markedly decrease respiration and produces antinociceptive effects in monkeys that are not antagonized by naltrexone. In studies on natural killer (NK) cell activity in mice, mirfentanil does not decrease NK activity at fully effective antinociceptive doses, whereas fentanyl, and most other fentanyl derivatives, markedly decreased NK activity. Collectively, these data suggest that compounds with strong antinociceptive effects, and without immunosuppressive effects, might be developed within this chemical family. Moreover, these studies demonstrate the necessity of characterizing the effects of novel phenylpiperidines under a wide range of conditions and in more than one species. Supported by USPHS DA05018, DA03573, and DA17-93-V-30137.

P-30
ANTI-RETROVIRAL EFFECTS OF AZIDOTHYMIDINE AND METHIONINE ENKEPHALIN USED IN COMBINATION

Steven Specter, *Nicholas Plotnikoff, Jeong-Im Sin, and Darlene Goodfellow
University of South Florida College of Medicine, Tampa, FL, and *University of Illinois at Chicago, Chicago, IL

The neuropeptide methionine enkephalin (Met-ENK - 1 or 3 mg/kg/dose) and AZT (7.5 or 15 mg/kg/dose) were used in a combined protocol for therapy of established murine retroviral infection. In the model used, Friend virus leukemia (FV), the drug combination was able to reduce mortality and splenomegaly. Of those animals that did not survive infection by FV, the combination increased mean survival time when compared to infected control mice or mice treated with AZT alone. However, Met-ENK used alone at 1 and 3 mg/kg/mouse had no effect in reducing morbidity or mortality due to FV. This suggested that Met-ENK had no direct antiviral effect at the concentrations used. In fact, mice treated with either single drug therapy or the combination still yielded virus in their spleen, even when splenomegaly was absent. In vitro studies using Met-ENK in FV infected *Mus dunni* cells confirm that the neuropeptide does not have direct anti-viral activity. However, spleen cells treated with Met-ENK in the presence of AZT reduced FV replication in culture. The data suggest that this combination may provide benefit in human retrovirus infections.

P-31
HOSPITALIZED DRUG USERS WITH HIV/AIDS: EFFECTIVE PROGRAM FOR DELIVERING MEDICAL CARE

Jo M. Leslie and Annie Umbricht-Schneiter*
The Johns Hopkins University School of Medicine, *National Institute on Drug Abuse, Division of Intramural Research, Baltimore, MD

In 1988 The Johns Hopkins Hospital opened a dedicated AIDS Unit consisting of 21 beds, staffed by infectious diseases faculty, medical residents, nursing, and social work personnel. The proportion of injecting drug users in the unit progressively increased. This resulted in management problems, including conflicts between patients and the health care team, staff concerns over personal security, and a number of discharges against medical advice (AMA). We hypothesized that a comprehensive program of staff and patient education would decrease these problems. The program consisted of 1) contractual rules for expected patient behavior, 2) individual and group interventions for drug use, 3) monthly education in addiction pharmacology for residents, and 4) continuing education for the entire health care team. This program has resulted in improved patient compliance, decreased AMA discharges, increased patient satisfaction, and

ACNP

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34th ANNUAL MEETING

SCIENTIFIC ABSTRACTS

SAN JUAN, PUERTO RICO

CARIBE HILTON

DECEMBER 11-15, 1995

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2014 Broadway, Nashville, TN 37203

President: David J. Kupfer
Program Committee Chair: Stephen H. Koslow

The meeting is held [illegible] the Graduate Continuing Education
in the Health Sciences at the University of Pittsburgh Medical Center and
Western Psychiatric Institute and Clinic

Poster Session I

RESPIRATORY AND DISCRIMINATIVE STIMULUS EFFECTS OF COMBINATIONS OF OPIOIDS AND BENZODIAZEPINES IN RHESUS MONKEYS

Charles P. France*, Lisa R. Gerak and Michael R. Brandt

Department of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70119

There is a high prevalence of benzodiazepine (BZ) use (either licit or illicit) among opioid abusers (e.g., Griffiths and Wolf, *J Clin Psychopharmacol*, 10:237-243, 1990) and in some populations BZs (e.g., flunitrazepam) are reported to be primary drugs of abuse. The purpose of the present study was to examine interactions between BZs and opioids in rhesus monkeys using measures of drug discrimination and ventilation.

When administered alone, both opioid (e.g., alfentanil) and BZ (e.g., lorazepam) agonists decrease ventilation (frequency [f], tidal volume [V_T] and minute volume [V_E]) in monkeys (n=4) breathing air or 5% CO_2; however, effects of morphine-like opioids increase monotonically up to doses that produce apnea whereas effects of BZs asymptote at V_E values between 60 and 90% (in air) of control. Acute pretreatment with lorazepam shifts the alfentanil dose-effect curves for f and V_E leftward, although the interactions are not greater than additive.

Four other monkeys receive 3.2 mg/kg/day of morphine and discriminate between 0.01 mg/kg of naltrexone and saline. Administration of naltrexone (> 0.0032 mg/kg) or termination of morphine treatment occasions complete ($\geq$90%) naltrexone-lever responding, and this effect is reversed by morphine and other μ agonists (e.g., alfentanil). BZ agonists neither substitute for naltrexone, attenuate naltrexone-lever responding in morphine-abstinent monkeys, alter the potency of naltrexone in producing drug-lever responding, nor alter the potency of alfentanil in attenuating drug-lever responding.

BZs are reported to attenuate some signs of opioid withdrawal and combinations of BZs and opioids are used routinely by some abusers, although the pharmacologic basis of this polydrug abuse is not clear. Results of the current study fail to demonstrate any enhanced toxicity of opioid/BZ combinations, in terms of effects on ventilation, and also fail to demonstrate significant discriminative (subjective) effects of BZs administered either alone or in combination with opioid agonists or antagonists in morphine-treated monkeys. Supported by DAMD17-93-V-3013 and USPHS Grants DA05018 and DA09157. Studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, Dept. of Health, Education and Welfare publication number (NIH) 85-23, revised 1985.

ASYMMETRY OF VISUAL INFORMATION PROCESSING AND SYMPTOMATIC HETEROGENEITY IN SCHIZOPHRENIA

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Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228

PAST TRAVEL AWARDEE POSTER

The deficit syndrome of schizophrenia is defined by the presence of primary, enduring negative symptoms, and preliminary studies have found these patients to have visual attentional impairments. Previous neurological, neuropsychological and PET studies have found convergent evidence of a possible dysfunction of a striato-pallido-thalamo-cortical circuit that involves the posterior parietal cortex in deficit schizophrenia. Posner et al. have found that patients with posterior parietal cortical lesions have contralateraly increased costs to invalid cues in a task of covert visual attention (CVA), and in acute schizophrenics they found the same pattern of response in the right visual field (RVF). Strauss et al., using this same task, found increased costs in the left visual field (LVF) in inpatient schizophrenics with prominent negative symptoms. We hypothesized that, compared to nondeficit schizophrenics, deficit patients will exhibit impaired CVA with increased costs in the LVF. We have studied 28 stable outpatients with schizophrenia (14 deficit, 14 nondeficit) and 20 normal volunteers with the CVA task using peripheral and central cues in a counterbalanced design. To ensure the validity of the paradigm, eye movements were monitored in all subjects with the infrared technique. Preliminary results with both types of cues were similar: Deficit patients were significantly slower in reaction time but did not show increased costs in the LVF. Nondeficit patients exhibited overall RVF slowing suggestive of impaired information processing greater in the left hemisphere. The RVF disadvantage originally described in acute schizophrenics as state-dependent may remain in a subgroup of patients characterized by the absence of enduring negative symptoms. In addition, correlations between CVA performance and other clinical and cognitive measures will be presented.

EFFECTS OF CONVULSANT AND ANTICONVULSANT AGENTS ON MEMORY IN SQUIRREL MONKEYS [1]

ERIC D. PAKARINEN [2], W. BRYANT FAUST AND JOSEPH M. MOERSCHBAECHER

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(Preliminary version, July, 1995)

Abstract

Pakarinen, Eric D., W. Bryant Faust and Joseph M. Moerschbaecher: Effects of Convulsant and Anticonvulsant Agents on Memory in Squirrel Monkeys. Prog. Newuro-Psychopharmacol. & Biol. Psychiat. 199X, X:

It has been reported that subconvulsive doses of convulsant agents such as strychnine and pentylenetetrazole can enhance memory in rodents studied under various behavioral procedures. The present study was designed to determine if similar results might obtain in squirrel monkeys. Responding by squirrel monkeys was maintained by food presentation under a repeated acquisition of behavioral chains procedure. Each subject acquired a different three-response chain each session. Sequence completions were reinforced under a fixed-ratio 5 schedule (FR 5) and errors produced a brief timeout. After the subject reached a predetermined acquisition criterion, the session was stopped and a 24 hr delay was interposed. Following the delay, the subject was retested on the same discrimination and retention was quantified as percent savings. When administered immediately after the subject reached the acquisition criterion, strychnine (0.0056 - 0.18 mg/kg) and pentylenetetrazole (0.32 - 42 mg/kg) had no effect on percent savings under the 24 hour delay. Similarly, the delta opioid agonist, BW373U86 (0.0056 - 3.2 mg/kg) [(±)-4-((α-R*)-α-((2S*,5R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-N,N-diethylbenzamide dihydrochloride], had little or no effect on percent savings following a 24 hr delay. This was true even at doses of BW373U86 which produced convulsions. In contrast, triazolam (1 - 1.8 mg/kg) decreased percent savings following the 24 hr delay. These results suggest that at subconvulsive doses, convulsant agents have little or no effect, while anticonvulsant agents such as triazolam can disrupt memory processes in squirrel monkeys.

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[1] A preliminary report of these data were presented at the annual Society fo Neuroscience Meeting in Miami Beach, Florida, in November, 1994.

[2] Present address of EDP: University of Michigan Department of Pharmacology, 1301 MSRB III, Ann Arbor, Michigan 48109-0632, phone: (313) 763-4085

Keywords: BW373U86, convulsant agents, delayed performance, pentylenetetrazole, memory, operant behavior, strychnine, squirrel monkeys, triazolam.

Abbreviations: benzodiazepine (BZD), H-Tyr-*D*-Thr-Gly-Phe-Leu-Thr-OH Hcl (DTLET), errors to criterion (ETC), fixed-ratio (FR), intracerebroventricular (i.c.v.), pentylenetetrazole, (PTZ).

Introduction

The behavioral properties of strychnine were first described by Lashley (1917) who reported that pretrial injections of strychnine facilitated the rate at which rats learned an alley maze. Since then, there have been many studies in which nonconvulsant doses of strychnine and other convulsant agents have been administered in an attempt to enhance learning or memory.

Typically, convulsant agents have been administered after acquisition has occurred and then retested after a specified delay to determine their effect on retention or memory (LeBoeuf and Peeke, 1969; Whishaw and Cooper, 1970). Under such conditions, memory has been reported to be enhanced (Alpern and Crabbe 1972, Crabbe and Alpern 1973).

The majority of such studies investigated the effects of convulsant agents in either rats or mice. In contrast, there are few studies reporting the effects of convulsant agents on memory in primates under stable behavioral baselines with operant procedures that are known to be sensitive to drug effects. Cook and Davidson (1968) reported that, at low doses (0.0625 - 0.25 mg/kg), strychnine increased overall correct responses and correct resonses following errors in four squirrel monkeys responding under a delayed matching-to-sample procedure. Thus, the objective of the present series of studies was to compare the behavioral effects of various convulsant agents on memory in squirrel monkeys. Specifically, our goals were to characterize the acute effects of convulsant and anticonvulsant agents on memory using the technique of repeated acquisition and delayed-performance. This technique has been previously used to characterize the effects of a variety of drugs on both learning and memory in monkeys (Thompson *et al.*, 1986; Auta *et al.*, in press).

Methods

Subjects

Eight adult female squirrel monkeys (*Saimiri sciureus*) served as subjects. Three of the subjects had a history of behavioral testing under a variety of reinforcement schedules, while the other five were experimentally naive at the initiation of behavioral training. Each subject was maintained at approximately 85% of its free-feeding body weight on a diet consisting of banana-flavored food pellets, monkey chow, fruit, peanuts, and vitamins. The banana pellets were earned during the experimental session, while the remainder of the diet was fed to each subject after each daily session. Water was continuously available in the home cages. Subjects were individually housed in a temperature and humidity-controlled room and kept on a 12 hour light-(7AM-7PM)-dark cycle.

Apparatus

During each session, the subject was seated in a Plexiglas chair (STC-300, BRS/LVE, Inc. Laurel, MD) with a water bottle attached to the left side. The chair was placed in front of a response panel inside a ventilated, sound-attenuating chamber (MEC-004, BRS/LVE, Inc. Laurel, MD). The response panel was equipped with three recessed keys (Coulbourn Instruments, Lehigh Valley, PA model 21-17) which were mounted 5 cm apart in a triangular configuration with the left and right keys at 30 degree angles relative to the center key. The reinforcer, a 190 mg Noyes banana-flavored food pellet (P. J. Noyes Company, Inc. Lancaster, NH), was delivered by a pellet dispenser (Gerbrands Corp., Arlington, MA, G5100 model D-1) into a well that was accessible through an aperture in the response panel measuring 4.7 cm H X 4.7 cm W. Behavioral events were scheduled and data recorded by a microprocessor (IBM PC, Armonk, NY), a printer (Epson LX-80, Torrance, CA) and a cumulative recorder (Gerbrands Corporation, Arlington, MA model C-4).

Procedure

A repeated acquisition and delayed performance procedure was used (cf., Thompson *et al.*, 1986). Each session was divided into three phases: acquisition, delay, and performance. During *acquisition* each subject was required to learn a different three-response chain each session in the presence of three different stimuli. The response keys were simultaneously illuminated by one of three colors; green, white, or amber. The subject's task was to press the correct key in the presence

of each color. For example, when the keys were green, the left key (L) was correct; when the keys were white with the house light on, the center key (C) was correct; when the keys were amber, the right key (R) was correct. After the completion of the chain there was a half-second flash of light in the feeder aperture which was paired with food delivery. After the feeder flash, the keylights remained off for one second before the chain reset. The same chain (in this case, Left-Center-Right or LCR) was repeated throughout the session. Food reinforcement was delivered under a fixed-ratio (FR 5) schedule (i.e., every fifth completion of the three-response chain produced a reinforcer). When the subject pressed an incorrect key (e.g., pressing L or R when C was correct), the error was followed by a 5-second timeout. During this time, the keylights were off and responses had no programmed consequence other than resetting the timeout interval. Responses during a timeout occurred infrequently after responding had stabilized. Errors did not reset the chain (i.e. non-correction). The chain was considered acquired when the subject completed a preset criterion of consecutive correct responses (30 - 108 depending on the subject). To establish a steady state of repeated acquisition (or learning), the three-response chain was changed from session to session. Six possible sequences of the three-response chain were used: LCR, CRL, RLC, LRC, CLR, and RCL. These were chosen on the basis of limits previously described (Thompson, 1973). When this acquisition criterion was met, the keylights were turned off and the *delay* (retention interval) began. After the delay, the subject was placed back in the experimental chamber and the session was accompanied by a tone (BRS/LVE Inc., Laurel, MD, SNA-001) which served as a discriminative stimulus for the *performance* phase. In this phase the subjects task was to perform the previously learned three-response chain. The performance phase was terminated after the same criterion of consecutive correct responses was met as in the acquisition performance phase. At the end of each session the subject was removed from the plexiglas chair and a short time later fed banana pellets to bring the total to 60 pellets/day. Sessions were normally conducted 5 days a week (Monday - Friday).

Drugs

Pentylenetetrazole, strychnine sulphate (Sigma Chemical Co., St. Louis, MO) and BW373U86, ((±)-4-((α-R*)-α-((2S*,5R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-N,N-

diethylbenzamide dihydrochloride) (Dr. R. McNutt, Burroughs Wellcome, Research Triangle Park, NC), were dissolved in 0.9% sterile saline. Triazolam (Upjohn, Kalamazoo, MI) was dissolved in 60% propylene glycol and 40% sterile water. Drugs and control (saline) injections were given in the gluteus muscle 15 minutes prior to the start of the experimental session at a volume of 0.5 ml/kg of body weight. Doses were tested in a mixed order. Higher doses were only tested once per week in order to minimize the development of tolerance or sensitivity to the drug. Lorazepam was administered in the event of a convulsion. At least one week of baseline sessions intervened between the each drug series. Generally, baseline sessions were conducted on Monday and Wednesday, drug sessions on Tuesdays and Fridays, and saline sessions on Thursdays.

Data Analysis

Under the delayed performance schedule, the degree of retention of the acquired response chain was quantified using a "percent savings" measure (Thompson *et al.*, 1986). Percent savings was calculated as follows: for a given response chain, the number of errors made before the acquisition criterion was met was compared to the number of errors made before the same criterion was met in the performance phase. Specifically, this comparison was calculated by subtracting the errors to criterion (ETC) in performance from the ETC in acquisition and then expressing this difference as a percentage of the ETC in acquisition. For example, if the subject made 40 errors before the acquisition criterion was met, but made only 10 errors before the same criterion was met in performance, the percent savings would be 75; [(40-10/40) X 100]. If retention was perfect (i.e., ETC in performance = 0), the percent savings would equal 100, whereas if there was no retention at all (i.e., ETC in performance $\geq$ ETC in acquisition), the percent savings would equal 0 or less. The data for each performance phase were also analyzed in terms of the overall response rate (total responses/minute, excluding timeouts) and the overall accuracy or percentage of errors [(errors)/(errors + correct) X 100] in the performance component.

The data for each subject were analyzed by comparing drug sessions with the range of saline sessions. A drug was considered to have an effect to the extent that the dose data fell outside of the saline range. In addition to these measures based on session totals, the within-session changes in responding were monitored by a cumulative recorder.

Results

The effects of pentylenetetrazole (PTZ) on the 24-hour delayed performance task are shown for two subjects in Figure 1. PTZ was administered immediately after the acquisition criterion was met and the subjects were retested on the same discrimination 24 hours later. Thus these experiments were designed to evaluate the effects of PTZ on memory storage. Under these conditions, PTZ had little or no effect on the rate of responding or percent errors in performance. Similarly, across the range of doses tested (0.32 - 42 mg/kg), PTZ had no effect on percent savings.

Insert Fig. 1 about here

The effects of strychnine on the 24-hour delayed performance task are shown in Figure 2. Like PTZ, strychnine had little or no effect on either the rate of responding or percent errors. Similarly, strychnine had little or no effect on retention (percent savings). Higher doses (>0.18 mg/kg) of strychnine were not tested because of convulsions.

Insert Fig. 2 about here

The effects of the delta opioid agonist BW373U86 on the 24-hour delayed performance task are shown in Figure 3. As was obtained with PTZ and strychnine, BW373U86 generally had little or no effect on either response rate, percent errors, or percent savings. Interestingly, BW373U86 had no effect on percent savings even at doses that produced convulsions (0.32 - 0.56 mg/kg). For example, in SQ M the first of two doses of 0.32 mg/kg produced a convulsion. Replication of this same dose in SQ M did not elicit a convulsion. However, a dose of 0.56 mg/kg of BW373U86 induced convulsions in all three subjects on each replication. Thus, tolerance failed to develop to the convulsive effects of 0.56 mg/kg across subjects under this dosing schedule.

Insert Fig. 3 about here

The effects of triazolam on the 24-hour delayed performance task are shown in Figure 4. Triazolam generally produced decreases in the rate of responding in SQ B only at the highest dose of triazolam (1.8 mg/kg). In monkeys SQ C and SQ D triazolam had little effect on the rate of responding. Triazolam increased percent errors only at the highest dose. In contrast to the other drugs tested, triazolam decreased percent savings to near zero at doses of 1 and 1.8 mg/kg in SQ D and at 1.8 mg/kg in SQ B and SQ C.

Insert Fig. 4 about here

Cumulative records showing a representative vehicle session and the highest dose of triazolam (1.8 mg/kg) that produced zero percent savings in SQ B are shown in Figure 5. As can be seen in the cumulative record, monkey SQ B made numerous errors early in the performance component. Moreover, it took the subject much longer to reach criterion than under control conditions.

Insert Fig. 5 about here

Discussion

In the present study drugs were administered immediately after the discrimination was acquired and its effects on storage were tested 24 hr later. In this situation the drug might be considered to be affecting storage because the drug is administered after learning has taken place and what has been learned is "encoded" or "stored" into memory (Thompson *et al.*, 1986; Moerschbaecher, 1989). In this type of manipulation, administration of a drug after acquisition has taken place can be made only at delays that exceed the duration of the drug's action. This is done in order to ensure that a different aspect of the drug's action is actually being measured, the drug must

have been largely eliminated prior to the performance phase. For example, if the delay was short and a long-acting drug was administered, it would essentially measure the drug's effect as if it were being administered shortly before performance or before retrieval. Thus both the length of the delay and the half-life of the drug must be taken into consideration when testing immediately after acquisition. Nonetheless, this particular aspect of the procedure is somewhat unique in that drug effects on storage may be studied. Other procedures designed to study memory such as DMTS, the delays which are investigated are usually too short (seconds - minutes) to allow such a manipulation. It should be noted that when studying the actions of a drug administered either after acquisition or prior to performance you are examining the effects of a drug on memory for a task acquired prior to drug administration. A disruption of memory obtained at either point could be further characterized as a retrograde amnestic effect (Moerschbaecher, 1989; Moerschbaecher, 1992). A final point at which a drug may be administered is prior to acquisition. Obviously, if the drug exerts an effect at this point we refer to it as either enhancing or disrupting learning rather than memory.

Neither strychnine nor pentylenetetrazole increased percent savings under the 24 hour delayed performance schedule. This is consistent with the results with strychnine in mice reported by Gordon *et al.* (1975). However, these results are in contrast to other reports that strychnine and pentylenetetrazole, when administered after a learning task has been completed, can enhance retention in rats and mice (McGaugh and Krivanek, 1970; Crabbe and Alpern, 1973; Leccese and Grant, 1980). Under the 24 hour delayed performance schedule the baseline percent savings was relatively high (about 80%) in three of the subjects. The other two subject's percent savings were about 60%. When initially trained, the baseline stabilized at about 50-60% savings for all subjects. With repeated training under this procedure the subjects's mean percent savings increased. Subsequently, the range for percent savings steadily increased. The subjects "learned to remember." Like the chain schedule that typically engendered low error levels, percent savings was relatively high in the majority of the subjects under this procedure. This could be attributed to either the delay (i.e., too short) or to task difficulty (i.e., too easy). As a consequence, detecting increases in percent savings or enhanced retention in the subjects might have been more difficult than if the baseline of percent savings had been lower throughout testing. Therefore, two manipulations that could be made to degrade the

baseline of percent savings might be to increase the delay (48 hours) or to increase task difficulty. Both or either of these manipulations would probably result in percent savings levels much lower than those engendered in the present studies. BW373U86, the non-peptide, systemically active, delta opioid agonist, was studied at two different delays in order to determine its effects on memory. The only other reports in the literature concerning the effects of delta opioid agonists on memory has been with deltorphin (Pavone *et al.*, 1990) and DTLET (Shiigi *et al.*, 1990) in mice. However, there were contrasting effects reported with both delta opioid agonists in these two studies. In the Pavone *et al.* (1990) study, deltorphin (a 7-amino acid peptide) was administered i.c.v. in two different inbred strains of mice after training in a one-trial inhibitory avoidance task. The retention of both strains of mice were improved by deltorphin administration. However, in the Shiigi *et al.* (1990) study DTLET (a 6-amino acid peptide) was administered intraperitoneally in mice before training on a passive avoidance learning task. DTLET (0.01 - 10 mg/kg) failed to enhance memory in the mice. Delta opioid receptor subtypes may exist in mice which are differentially sensitive to the effects of deltorphin and DTLET. Another reason that Shiigi *et al.* (1990) might not have detected memory enhancing effects of DTLET might be that the peptide was broken down systemically by peptidases such that very little of the drug could cross the mouse blood-brain barrier. Clearly, there were many differences between the present and previous studies (i.e., species, route of administration, drug). A consistent determination of whether the delta opioid receptor is tonically active in memory processes remains to be determined.

Tolerance developed to the convulsant effects of BW373U86 in the squirrel monkeys. Tolerance to the convulsant effects of BW373U86 has been reported in mice receiving systemic doses (3.2 - 100 mg/kg) of the delta opioid agonist (Comer *et al.*, 1993) and in squirrel monkeys (Pakarinen *et al.*, under review). The subjects were initially exposed to the range of doses of BW373U86 under the 24 hour delay. For example, at the 24 hour delay, 0.56 mg/kg of BW373U86 produced convulsions in all three monkeys.

In summary, BW373U86 had little or no effect on storage even at doses that produced convulsions. These results suggest that BW373U86 can produce convulsions but without the retrograde amnestic effects that have been reported to occur with other convulsants or shock-induced

convulsions (Pearlman *et al.*, 1961; Bohdanecky *et al.*, 1968; Misanin *et al.*, 1968). This represents an interesting dissociation between convulsions and amnesia.

Snead and Bearden (1980) reported that enkephalins, which are delta opioid agonists, might be involved in the production of absence seizures. This type of seizure is characterized by impaired consciousness with the individual appearing to be in a trance during which time there is a temporary suspension of mental functions (Mattson *et al.*, 1978). Snead and Bearden (1980) reported that in rats, anticonvulsant drugs specific for absence epilepsy (ethosuximide, valproic acid, and trimethadione) abolished the electrical seizure activity produced by leucine enkephalin given i.c.v. Naloxone also abolished this effect. However, drugs used in the treatment of tonic-clonic epilepsy such as phenobarbital and phenytoin had no antagonistic effect. These results suggested the delta opioid receptor might play a role in nonconvulsive seizures disorders. Even though BW373U86 produced convulsive seizures at high doses, the possibility cannot be ruled out that at lower doses nonconvulsive seizures might have been occurring. It would be of interest to determine the effects of BW373U86 in combination with anticonvulsants specific for absence epilepsy. BW373U86 had little or no effect on delayed performance at a 24 hour delay even after drug-induced convulsions. Whereas under a repeated acquisition procedure, BW373U86 disrupted learning (Pakarinen *et al.*, 1995). Thus, BW373U86 might exert a selective desruptive effect on learning but not memory.

The relatively short-acting triazolo-benzodiazepine, triazolam, was tested to ensure that the delayed performance procedure in squirrel monkeys was sensitive to the amnestic effects of drugs. Triazolam was chosen primarily because the triazolo-substituted compound has been shown to produce amnestic effects following their oral administration in both humans (Weingartner *et al.*, 1992; Berlin *et al.*, 1993; Roache *et al.*, 1993) and Old World monkeys (Moerschbaecher *et al.*, 1987; Moerschbaecher, 1989; Auta *et al.*, in press). Triazolam produced retrograde amnestic effects in squirrel monkeys consistent with the effects reported in Old World monkeys (Moerschbaecher *et al.*, 1987). These data suggest that the delayed performance procedure was sensitive to the effects of drugs on memory in monkeys.

The focus of these studies have been on the effects of drugs on learning and memory. Reports in the literature suggest that administration of low doses of convulsant agents such as PTZ and

strychnine can enhance memory tasks (Bovet *et al.*, 1966; Krivanek and McGaugh, 1968; Alpern and Crabbe, 1972; Crabbe and Alpern, 1973). These reports have used non-operant techniques to study drug effects on memory in rodents. In some cases, subjects were administered drugs before training to determine their effects on learning. In other cases the subjects were given relatively few trials, then administered drugs to determine their effect on retention or memory. The predictive utility of mazes and passive avoidance tests as rodent models to determine the effects of drugs on memory in man might be questioned. However, until a clinically proven prototypical agent becomes available this question will remain unanswered. Results from the present series of studies are in contrast to the numerous non-operant reports that low doses of convulsants can enhance memory in rodents. These differences between squirrel monkeys and rodents may have significant implications concerning the neuropharmacology of memory.

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Running Title: Drug Effects on Memory

FIGURE LEGENDS

Figure 1. Effects of varying doses of pentylenetetrazole on the overall response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Points at S indicate the mean and range of at least eight sessions that were preceded by a saline injection. The points with the vertical lines in the dose-response curves indicate the mean and range for two determinations. The points without vertical lines indicate either a single determination or an instance in which the range is encompassed by the point. When the response rate was less than 5 resps/min, no data point for that dose was shown for percent errors.

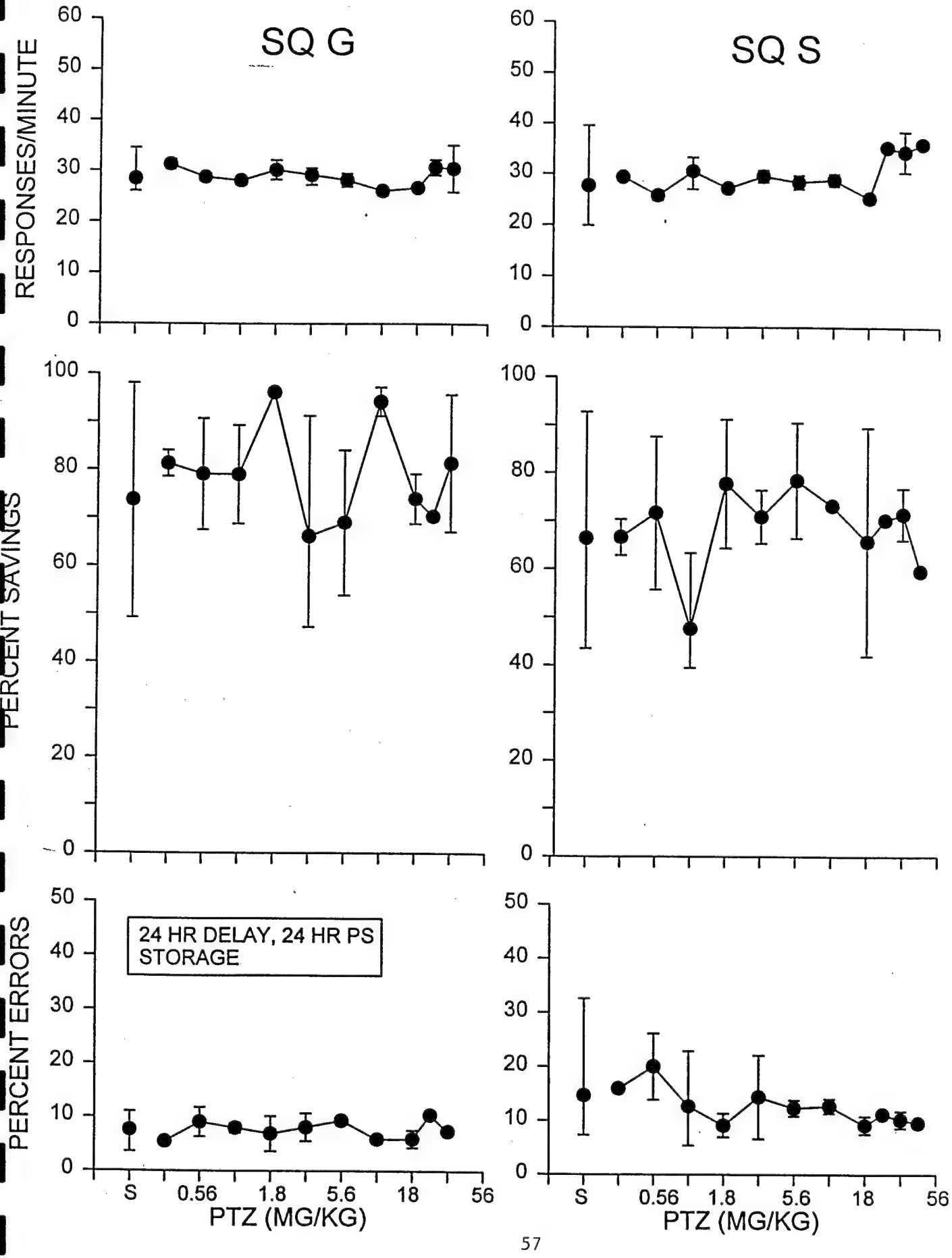
Figure 2. Effects of varying doses of strychnine on overall response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Other details are the same as in Figure 1.

Figure 3. Effects of varying doses of BW373U86 on overall response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Convulsant doses are indicated on the percent savings panel by an asterisk. Other details are the same as in Figure 1.

Figure 4. Effects of varying doses of triazolam on overally response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Other details are the same as in Figure 1.

Figure 5. Cumulative response records for SQ B showing the pattern of responding during a saline session and following a dose of triazolam given 24 hours prior to the delayed performance. The response pen stepped up with each correct response and was deflected downward with each completion of the three-response chain. Reinforcement was delivered with every fifth deflection of the response pen. Errors were indicated by deflections of the lower event pen.

Figure 1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SQ S
RESPONSES/MINUTE
PERCENT SAVINGS
PERCENT ERRORS
24 HR DELAY, 24 HR PS STORAGE
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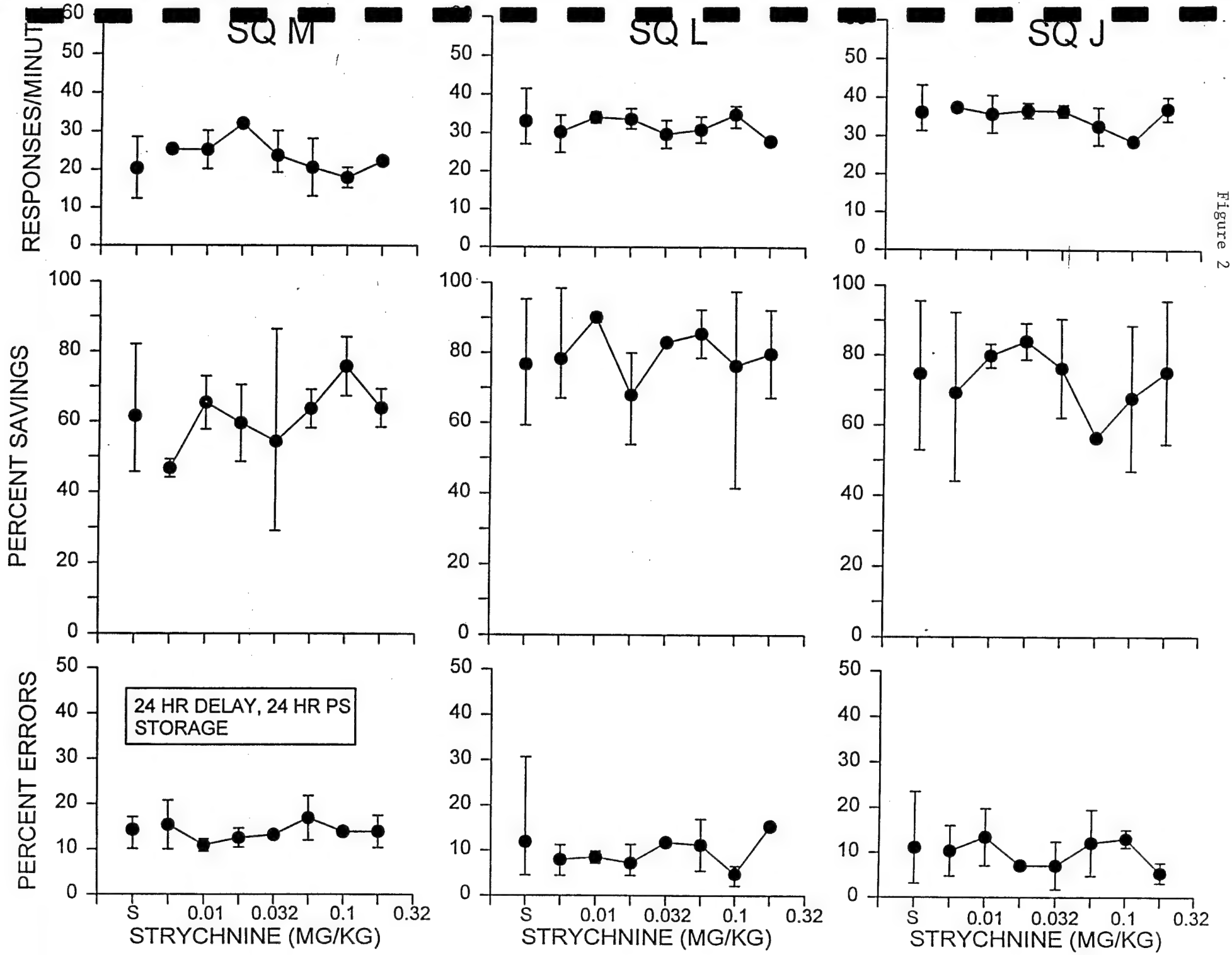
5.6
18
56
PTZ (MG/KG)


SQ M
SQ L
SQ J
RESPONSES/MINUT
PERCENT SAVINGS
PERCENT ERRORS
24 HR DELAY, 24 HR PS STORAGE
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10
20
30
40
50
60
80
100
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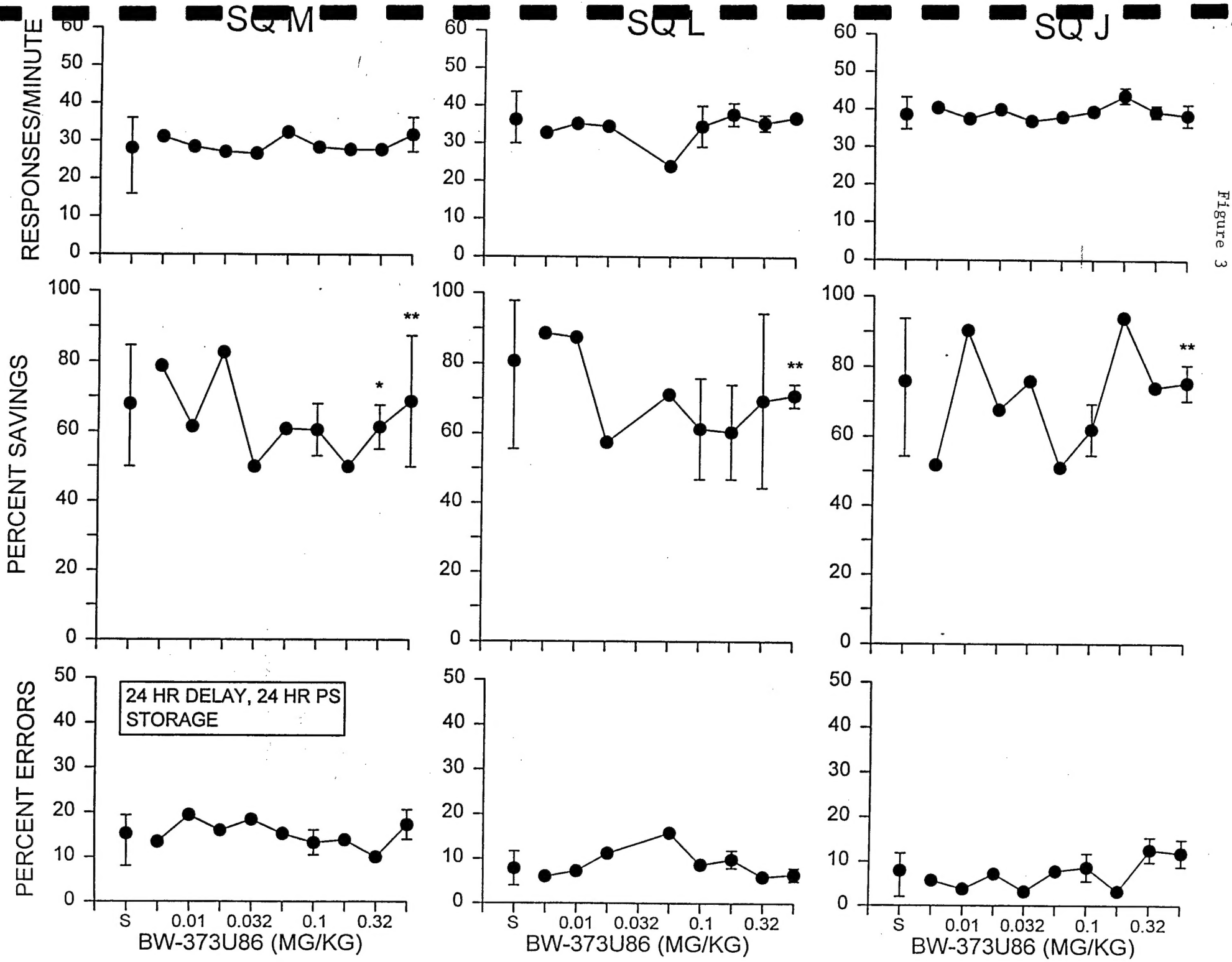
0.032
0.1
0.32
STRYCHNINE (MG/KG)

Figure 2


SQM
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RESPONSES/MINUTE
PERCENT SAVINGS
PERCENT ERRORS
24 HR DELAY, 24 HR PS STORAGE
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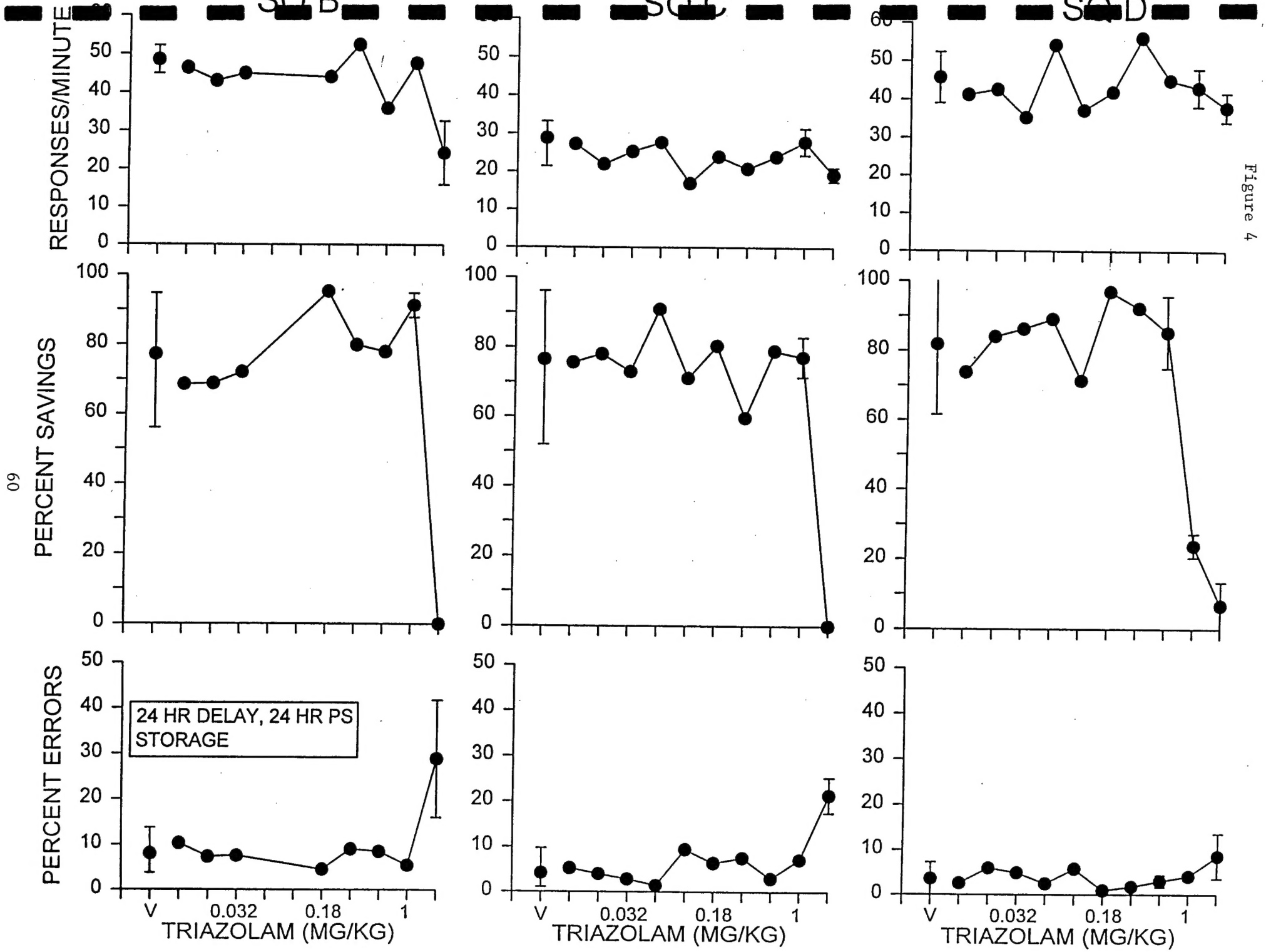
0.032
0.1
0.32
BW-373U86 (MG/KG)

Figure 3


SQB
SQC
SQD
RESPONSES/MINUTE
PERCENT SAVINGS
PERCENT ERRORS
24 HR DELAY, 24 HR PS STORAGE
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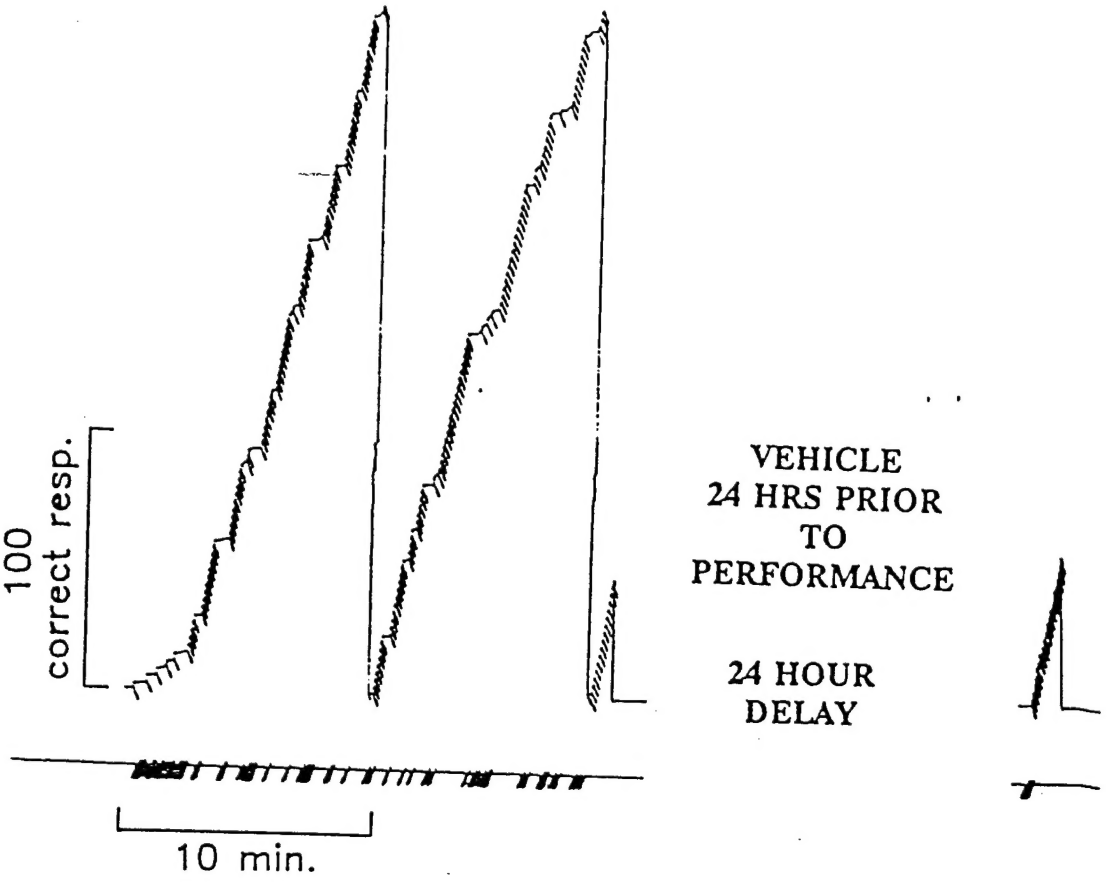
0.18
1
TRIAZOLAM (MG/KG)

Figure 4

Figure 5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correct resp.
10 min.
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24 HRS PRIOR
TO
PERFORMANCE
24 HOUR
DELAY
TRIAZOLAM
1.8 MG/KG
24 HRS PRIOR
TO
PERFORMANCE
24 HOUR
DELAY
ACQUISITION
PERFORMANCE

FOOTNOTES TO THE TITLE PAGE

[1]This work was sponsored in part by the Department of the Army, Cooperative Agreement DAMD 17-93-V-3013. This does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred.

[2]Animals used in these studies were maintained in accordance with the Committee on the Use and Care of Animals, Louisiana State University Medical Center and the guidelines of the Committee on Care and Use of Laboratory Animals Resources, National Research Council, Department of Health, Education, and Welfare Publication Number (National Institutes of Health) 85-23, revised 1985.

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